

# The role of the peptides in enzymes at the origin of life

Søren Toxvaerd

*DNRF centre "Glass and Time," IMFUFA, Department of Sciences,  
Roskilde University, Postbox 260, DK-4000 Roskilde, Denmark*

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The peptides in biosystems are homochiral polymers of L-amino acids, but racemise slowly by an active isomerization kinetics. The chemical reactions in biosystems are, however, reversible and what racemises the peptides at the water activity in the biosystems can ensure homochirality at a smaller activity. Here we show by a thermodynamics analysis and by comprehensive Molecular Dynamics simulations of models of peptides, that the isomerization kinetics racemises the peptides at a high water activity in agreement with experimental observations of aging of peptides, but enhances homochirality at a smaller water activity. The hydrophobic core of the peptide in an enzyme can ensure homochirality at a low water activity, and thus the establishment of homochirality at the origin of life and aging of peptides and dead of biosystems might be strongly connected.

## I. INTRODUCTION

Biosystems consist of chiral polymers, where the building units are L- amino acids and D-carbohydrates. Another general behavior of the polymers in the cells, the peptides and DNA, is that they perform "higher-order" conformational structures of the homochiral units [1–3]. But neither the peptides, nor DNA in the cells [4–6] are long time stable. The L-amino acids in aqueous solutions [7, 8], and the homochiral peptides in the cells racemise slowly [9–13]. The racemisation of the peptides in biosystems is associated with an increased concentration of water molecules in the peptides and lost  $\beta$ -sheet structure [10]. The water activities in biosystems differ with respect to the extracellular- and intracellular water activity. Whereas the extracellular fluid in biosystems can be characterized as a rather ideal solution ("saline solution"), the intracellular fluid (cytosol) does not behave as a diluted aqueous solution [14]. The origin of homochirality in the biosystems is unknown, but since the homochirality of the peptides degenerates in time, it must have been established at a condition, which deviates from the physicochemical condition in biosystems.

## II. THE PHYSICOCHEMICAL CONDITION FOR STABLE HOMOCHIRALITY IN AQUEOUS SOLUTIONS

A pure racemic mixture of D- and L-molecules can separate in homochiral domains if the enthalpy gain, due to the chiral discrimination, is bigger than the entropy of mixing, just as in the case of Pasteur's experiment [15], but now in a fluid state. A racemic mixture of chiral molecules and without an isomerization kinetics will separate in homochiral subdomains by molecular diffusion. This more ordered state can spontaneously be obtained from a prebiotic state with racemic portions of the building units (sugars and amino acids) provided that the re-

action Gibbs free energy,  $\Delta_r G = \Delta_r H - T\Delta_r S < 0$ , is negative.

The separation to the homochiral states has a negative reaction entropy,  $\Delta_r S < 0$ . If the temperature,  $T$ , times the reaction entropy,  $T\Delta_r S < 0$  by a separation from the racemic state to the two homochiral states is less negative than the corresponding reaction enthalpy,  $\Delta_r H < 0$  it will ensure a negative reaction Gibbs free energy. A simple "ideal mixture" estimate of the entropy contribution to the reaction Gibbs free energy gives [16]

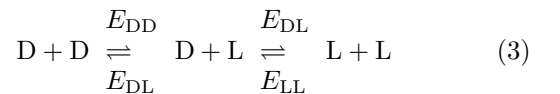
$$T\Delta_r S \approx -RT \ln 2 \approx -2 kJmol^{-1} \quad (1)$$

at moderate and biological relevant temperatures, and the system will phase separate into homochiral domains, if the gain of reaction enthalpy given by a "chiral discrimination" is strong enough, i.e.

$$\Delta_r H < -2 kJmol^{-1}. \quad (2)$$

This fundamental physicochemical mechanism has recently been observed for spontaneous phase separation in a fluid mixture into coexisting fluid domains of homochiral molecules [17].

An active isomerization kinetics in a racemic mixture with a reaction enthalpy,  $\Delta_r H < T\Delta_r S$  can, however, also ensure the establishment of only one homochiral domain [18]. Biochemical reactions are typically bimolecular. The bimolecular isomerization kinetics of amino acids is



between the two chiral species, D and L. The activation energy,  $E_{DL}$ , for a DL-collision which may convert a D-molecule into a L-molecule or *vice versa*, is, in a condensed racemic fluid, less than the corresponding activation energy,  $E_{DD} = E_{LL}$ , thus allowing a conversion

of one of the molecules in the collisions. The inequality,

$$E_{DL} < E_{LL} = E_{DD}, \quad (4)$$

accounts for the chiral discrimination with a lower potential energy for a homochiral pair of molecules, than for an enantiomer pair, corresponding to a sufficient strength of the chiral discrimination. Let the rate constants,  $k_{DL}$  and  $k_{LL} = k_{DD}$ , be given by Arrhenius expressions:  $k_{DL} = A_{DL} \exp^{E_{DL}/RT}$ ,  $k_{LL} = A_{LL} \exp^{E_{LL}/RT}$ . The difference in activation energies is proportional to the (local) chiral discrimination,  $E_{DL} - E_{LL} \propto \Delta_r H(x)$ , where a local gain in enthalpy,  $-\Delta_r H(x) > 0$ , by a conversion of the stereo configuration is a function of the *local* composition,  $x(\mathbf{r})$  of the chiral particles nearby the position  $\mathbf{r}$ .

The strength of chiral discrimination depends on how well the molecules fits into a chiral structure and is given by the complex potential function between a molecule and its chiral neighbours. Primarily  $\Delta_r H(x)$  depends on the excess number of homochiral neighbours by a change of a configuration. Consider a simple example: let a molecule (or a chiral unit in a peptide) at the position  $\mathbf{r}$  be in e.g. a L configuration before it is activated to a (intramolecular) transition state configuration. It will with a Boltzmann probability choose the configuration with lowest potential energy. A molecule in a liquid mixture has typically eleven to twelve nearest neighbours. Let e.g. five of them be in a D configuration, four of the neighbours in a L configuration and three neighbours be indifferent water molecules. It corresponds to a local racemic composition near  $\mathbf{r}$  of thirteen molecules with an equal amount of D and L configurations before the activation of the L molecule at  $\mathbf{r}$ . But due to the local excess of D molecules around the activated molecule, it will most likely turn into a D configuration by which the system tends to a lower energy, but now with an excess of D configurations. Thus a strong chiral discrimination together with an isomerization kinetics will ensure a separation of a racemic mixture on a molecular level and tend to order the chiral units in homochiral clusters and subdomains. The same kinetics for homochirality must also be valid for the chiral units in the peptides, where the symmetry break is caused by the chirality in the domain of nearest chiral units.

The homochiral dominance are obtained by, what could be expressed as *self-stabilizing chance* [16]. The deviation from a racemic mixture is self-stabilizing, because homochiral clusters- or sub domains catalyze their own growth by the isomerization kinetics, which mainly takes place in the interface, whereas the chiral discrimination inside the homochiral domains slows down the kinetics and the conversions of the configurations, which always are unfavorable. Still one needs to explain the observed dominance, since the kinetics seems only to enhance the separation, but *it does not favour one of the chiral species*. The break of symmetry on a macroscopic scale, and the establishment of one stable homochiral domain will appear, when one of the homochiral domains encap-

sulates the other domain [18, 19].

The simple carbohydrates and amino acids are soluble in water. The chiral discrimination in an *ideal* solution of amino acids or simple carbohydrates in water with the mole fraction  $x_w$ , is reduced proportional to the number of chiral neighbours, and is approximately

$$\Delta_r H(aq) \approx (1 - x_w) \Delta_r H. \quad (5)$$

The chiral discrimination near a surface,  $\Delta_r H_{surf.}(aq) \approx 0.5(1 - x_w) \Delta_r H$ , is further reduced by a factor of the order two due to halving of the number of nearest neighbours, and with the catastrophic result, that a chiral purification is only possible for an extreme high concentration of the chiral units or an extreme high strength of chiral discrimination, no matter where in the fluid. It explains why the presence of water molecules affects the quaternary structure of a peptide and reduces the chiral order [10, 12].

The stability of chiral order in presence of water can be obtained for ideal mixtures. The equilibrium constant  $K$  for a diluted solution of e.g. L-chiral molecules or a peptide with L- amino units in equilibrium with a small fraction of its enantiomer by an active isomerization kinetics, is

$$K = \frac{x_L x_D}{x_L^2} = \frac{x_L(1 - x_L - x_w)}{x_L^2} \\ = \frac{k_{LL}}{k_{DL}} = \exp^{(E_{LL} - E_{DL})/RT} = \exp^{x_w \Delta_r H/RT}. \quad (6)$$

The equation determines the mole fractions  $x_L$  and  $x_D$  as a function of  $x_w$  and  $\Delta_r H$  for an aqueous solution of chiral molecules in equilibrium with a small fraction of its image molecules by an isomerization kinetics.

### III. MOLECULAR DYNAMICS SIMULATIONS OF PEPTIDES IN AQUEOUS SOLUTIONS AND WITH ISOMERIZATION KINETICS

The chiral discrimination,  $\Delta_r H$ , depends on how well a chiral molecule, or a mirror image of the molecule, packs with other chiral molecules (e.g. amino acids or carbohydrates). The net energy difference is given by complex potential functions. But since it is the net gain of energy which gives the strength, it can be obtained by an energy function, which ensures a correct gain of energy from the interactions between the molecule and its neighbour molecules. Here we simulate, by Molecular Dynamics (MD), such systems of peptides of "united atom" units with chiral energy differentiation.

The system consists of  $N = 40000$  Lennard Jones (LJ) particles in a cubic box with periodical boundaries [20]. The MD simulations are performed with the central difference algorithm in the leap-frog version, and the forces for particle distances greater than  $r_{cut}$  are ignored. There are different ways to take the non-analyticity of the force at  $r_{cut}$  into account. The most stable and energy conserving way is to cut and shift the forces (SF), [21] by

which one avoids a nonphysical force gradient at the cut. The SF-MD simulations are performed for a temperature  $T=1.00$  and a density  $\rho=0.80$ , which corresponds to a condensed liquid at a moderate ("room") temperature.

The peptide chains are constructed by linking LJ-units together by reflecting the LJ potential between two neighbour units in the chain at the potential minimum [22]. This anharmonic bond potential is LJ-like and ensures a smooth interaction of an "amino acid unit" in the peptide with the water particles, as well as with the other units in the chain molecule.

### A. Potentials for hydrophobic and hydrophilic behaviour

The structure of a fluid is mainly determined by the forces within the first coordination shell of nearest neighbour particles [23], and it is also the short range attractive forces which determines the strength of the chiral discrimination. At the state point  $(T, \rho) = (1.00, 0.80)$  the range of the first coordination shell (fcs) in the LJ system is  $r_{\text{frc}} \approx 1.55$ .

The range of attraction for homochiral pairs (DD) or (LL) is taken to be equal to the radius of the first coordination shell,  $r_{\text{cut}}(\text{DD}) = r_{\text{cut}}(\text{LL}) = r_{\text{frc}} = 1.55$ , by which one obtains a maximum attraction between homochiral pairs. A smaller mean energy for a racemic composition is then achieved by using a smaller range of attraction between enantiomers. The range of attraction between two enantiomers is taken to be  $r_{\text{cut}}(\text{DL})=1.35$ , by which the mean potential energy difference,  $\Delta u$ , between a racemic mixture and a homochiral fluid is determined to be  $\approx T \ln 2$ . (The MD is for canonical ensemble dynamics (NVT), where the chiral discrimination is given, not by an enthalpy difference, but with the corresponding potential energy difference.) Consistent with this choice, MD simulations of a racemic mixture without isomerization kinetics separate slowly into a D- and a L- reach domains for a smaller range,  $r_{\text{cut}}(\text{DL}) < 1.35$ , as one shall expect from thermodynamics considerations [21].

Molecules can be sorted into hydrophilic- and hydrophobic molecules according to their solubility in water. But the word "hydrophilicity" is perhaps a bit misleading, since there is only one molecule which is hydrophilic, and this is  $\text{H}_2\text{O}$ . And although all simple carbohydrates and amino acids are soluble in water for small or moderate concentrations, they separate at higher concentrations. The strong hydrophilicity between LJ "water" molecules (W) is achieved by using  $r_{\text{cut}}(\text{WW}) = 1.55$ , i.e. a strong mean attraction between pairs of water molecules, equal to the strong attraction between two homochiral units.

The hydrophilicity or hydrophobicity between a water molecule and a chiral molecule -or chiral unit in a peptide, is also monitored by the range of attraction. A hydrophobic D or L unit have a small attraction (small

water activity) to a water molecule. This hydrophobicity is achieved by using a cut  $r_{\text{cut}}(\text{DW}) = r_{\text{cut}}(\text{LW}) \leq 1.35$ , i.e. less or equal to the attraction between two enantiomers, whereas a more hydrophilic unit in the peptide have an attraction  $r_{\text{cut}}(\text{DW}) = r_{\text{cut}}(\text{LW}) \geq 1.40$ , which corresponds to a higher water activity.

We have constructed chains with different numbers,  $N_p$ , of LJ-units and in aqueous solutions with  $N_w = N - N_p$  water molecules. The hydrophobic peptides have a compact globular form with a low fraction of water molecules in the peptides, whereas the chains swell up at a higher water activity.

### B. Aqueous solutions of chiral molecules with isomerization kinetics

The isomerization kinetics is performed as described in Ref. 6. A particle, No.  $i$ , at time  $t$  is activated by a collision with one of its nearest neighbours, No.  $j$ , if the potential energy at the collision

$$u_{ij}(t) \geq E, \quad (7)$$

where  $E$  is equal to  $E_{\text{DD}} = E_{\text{LL}}$ , if  $j$  has the same chirality as  $i$ , and equal to  $E_{\text{DL}}$  if not.

The (total) potential energy of particle No  $i$ , at the time where it collides with  $j$ , is

$$u_i(t) = \frac{1}{2} \sum_k u_{ik}(t) \quad (8)$$

for the sum over interactions with  $i$ 's nearest neighbours. Correspondingly the potential energy of  $i$  is

$$\tilde{u}_i(t) = \frac{1}{2} \sum_k \tilde{u}_{ik}(t), \quad (9)$$

if  $i$ 's chirality is changed. The chirality of particle No.  $i$  is then changed in the traditional way by a Boltzmann probability from  $\Delta_r u = \tilde{u}_i(t) - u_i(t)$  [18].

According to the thermodynamics, the strength of the chiral discrimination can be obtained from the activation energies,  $E_{\text{DL}}$  and  $E_{\text{LL}} = E_{\text{DD}}$ . The chiral discrimination in favor of homochirality has to be

$$-\Delta_r H = E_{\text{LL}} - E_{\text{DL}} \geq T \Delta_r S = T \ln 2. \quad (10)$$

The MD systems perform symmetry breaks with chiral purification for  $E_{\text{LL}} - E_{\text{DL}} = E_{\text{DD}} - E_{\text{DL}} \geq 2T$ , in agreement with the thermodynamics (ideal mixture) estimate [16]. The isomerization kinetics in the next Section are for  $E_{\text{LL}} - E_{\text{DL}} = E_{\text{DD}} - E_{\text{DL}} = 8T - 5T$ .

## IV. PEPTIDES IN AQUEOUS SOLUTIONS

The simulations of amino acids and peptides in aqueous solutions confirm the thermodynamic derivations in Section II. It is only possible to obtain a symmetry break

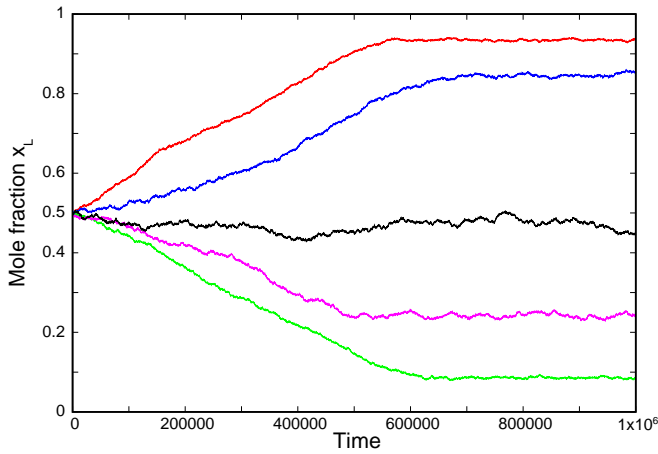


FIG. 1. Mole fraction  $x_L(t)$  as a function of time (steps) in aqueous solutions of simple chiral molecules (e.g. amino acids) with isomerization kinetics and a strong chiral discrimination. The start configurations are racemic ( $x_L(0) = 0.5$ ). Red curve is  $x_L(t)$  for a solution with a mole fraction of water molecules  $x_w = 0.025$ ; green curve:  $x_w = 0.05$ ; blue curve:  $x_w = 0.125$ ; magenta curve:  $x_w = 0.2$  and black curve:  $x_w = 0.25$ . The isomerization kinetics does not favor one of the chiral conformations, and a L or D dominance is obtained by chance with equal probability, as demonstrated by the examples in the figure. The equilibrium mean fractions after the symmetry breaks are given by Eq. (6).

and chiral dominance in the MD system for concentrated solutions of chiral molecules, here with  $x_w \leq 0.20$  (Figure 1). But although all simple carbohydrates and amino acids are soluble in water for small or moderate concentrations, they separate and crystallize at higher concentrations. Biosystems are, however in a fluid state, and there seems to be only one possibility for a selforganised chiral purification and maintenance of homochirality in aqueous solutions: *peptides with a hydrophobic core*.

We have constructed chains with different numbers,  $N_p$ , of chiral hydrophobic or hydrophilic units, and in aqueous solutions. The hydrophobic peptides have a compact globular form with a low fraction of water molecules in the peptides, whereas the chains swell up at a higher water activities. There are also differences in the conformations for chains with a racemic (random D- and L-) and a homochiral composition of hydrophobic units. The racemic peptides (Figure 2, green points) have a significant higher water content than the homochiral peptides (red points), and it exceeds  $x_w \approx 0.20$  for the racemic peptides with chain lengths less than  $N_p \approx 400$ . The blue points are for hydrophobic peptides with isomerization kinetics. These properties shown in Figure 2 are in qualitative agreement with the experimental observation of a peptide after aging with loss of homochirality [10].

Figure 3 shows the evolution of chiral dominance for different sizes of the hydrophobic peptides. The presence of water with a mole fraction  $x_w \geq 0.20$  makes the ho-

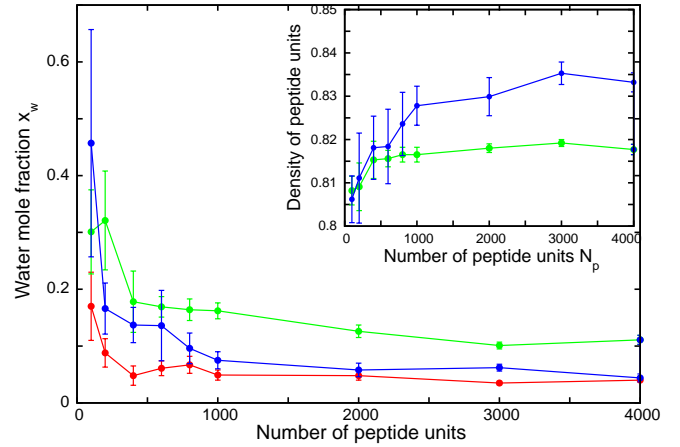


FIG. 2. Mole fraction,  $x_w$ , of water molecules in the sphere with radius equal to the radius of gyration for peptides with  $N_p$  hydrophobic units with strong chiral discriminations. The red points are for homochiral peptides and the green points are for racemic peptides. The blue points are for hydrophobic peptides with isomerization kinetics and they have only chiral dominance for  $N_p \geq 400$ . The inset shows the corresponding densities of the units and for racemic peptides (green) and peptides with isomerization kinetics (blue). (The uncertainties are obtained from ten independent simulations.)

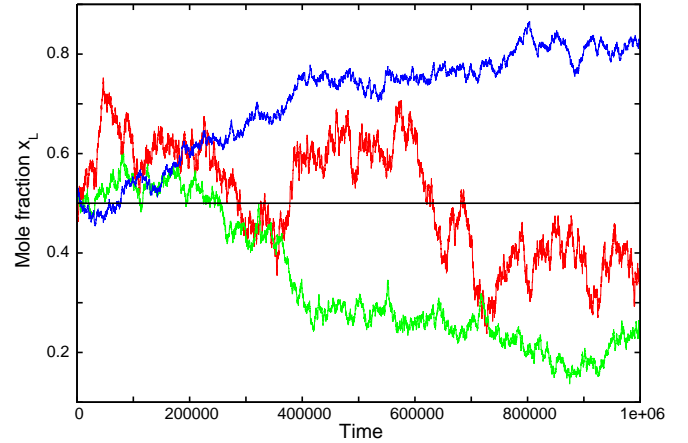


FIG. 3. Mole fraction  $x_L$  of L-units in chains with  $N_p = 400$  (red),  $N_p = 1000$  (green) and  $N_p = 2000$  (blue) chiral units, and with isomerization kinetics. The chains have a random and racemic distribution at the start.

mochiral state unstable (Figure 1), and favor the racemic state according to the thermodynamics, and the results in Figure 2 (blue line and points) shows that small peptides with hydrophobic units and isomerization kinetics contain water molecules with a fraction, which exceeds this stability limit. In accordance with these results we observe, that peptides of hydrophobic units with isomerization kinetics and chiral discriminations can not maintain homochirality when  $N_p \leq 400$ , and Figure 3 demon-

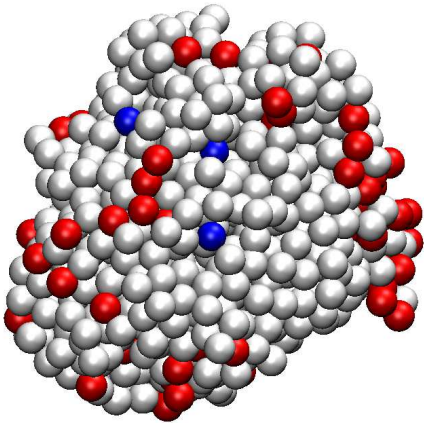


FIG. 4. A peptide of  $N_p = 1000$  hydrophobic units and with isomerization kinetics. The peptide is compact and rather homochiral ( $x_L=0.15$ ) with dominating D-units (white). The L-units (red) are mainly located in the water-peptide interface. There are 15 water molecules (blue), mainly located in pockets of the compact peptide and with a few in the interior.

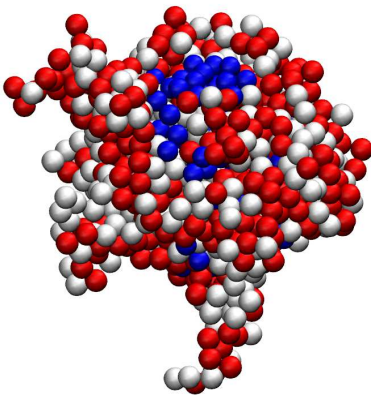


FIG. 5. The peptide with  $N_p = 1000$  units at a higher water activity, and with isomerization kinetics. The composition (L/D=red/white) is now  $\approx$  racemic and the concentration of the water molecules are increased to 39.

strates this fact. The fraction of the L-state,  $x_L$ , for a small peptide of  $N_p = 400$  units (red line) fluctuates, but remains racemic in mean. For longer chains one obtains, however, a symmetry break to a state with a dominating chirality, either to a D-state ( $N_p = 1000$ : green), or to a L-state ( $N_p = 2000$ : blue).

The hydrophobic peptide with  $N_p = 1000$  units, with  $x_L(t)$  shown in Figure 3 (green line) is rather compact

and globular. The peptide was simulated over a longer time period, where it maintains the chiral D dominance. The configuration at the end of the simulation is shown in Figure 4. The strong hydrophobicity corresponds to a small water activity. If this peptide, however is exposed to a higher water activity it swells up with a bigger contents of water in the core, and the peptide loses its homochirality (Figure 5).

We have performed many simulations, for different strengths of hydrophobicity, water activity and chiral discrimination ( $E_{DL} - E_{LL}$ ), and they conform the result, shown in the figures, that spontaneous chirality is only achieved for sufficient strength of chiral discrimination,  $E_{DL} - E_{LL} = E_{DL} - E_{DD} \geq 2$ , and for hydrophobic peptides with a molfraction of water less than  $x_w \approx 0.20$ , in accordance with the thermodynamics for obtaining spontaneous homochirality.

## V. PERSPECTIVE

The strength of chiral discrimination depends on how well a molecule packs with a chiral molecule or with the mirror form of the molecule. Normally one associates it with how well a molecule fits with a copy of itself, compared with how well it fits with its mirror image molecule. But there is also chiral discriminations between different amino acids [24, 25] and between simple carbohydrates and amino acids [26–28]. There is a chiral discrimination between a simple carbon hydrate, D-Glyceraldehyde, and the amino acid L-Serine [26, 27], which can explain the overall dominance of D-carbohydrates and L-amino acids in biosystems.

The present investigation indicates that the function of the peptides in enzymes, at the origin of homochirality and life, first were to stabilize homochirality of the units in the hydrophobic core of the peptides, whereby they act as the "backbones" with a stereo specific surfaces for the homochiral purification of carbohydrates and amino acids by the isomerization kinetics. The chemical reactions in biosystems are reversible, and what ensures homochirality at low water activity will racemise at higher water activity. The water activity at the establishment of homochirality must necessary have been somewhat smaller than the aqueous cytosol solutions in the cells. The smaller water activity can be obtained by a more concentrated solutions of amino acids and carbohydrates, and at somewhat higher ionic concentrations than the ionic concentrations in the cytosol. Experiments on such aqueous solutions can reveal, whether it is possible to obtain spontaneous chiral purification and to maintain homochirality in presence of peptides with hydrophobic cores.

## VI. ACKNOWLEDGMENT

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